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SCP-Related Peptides From Bivalve Mollusks: Identification, Tissue Distribution, and Actions

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Abstract. The SCPs[3] are a small peptide family, characterized in gastropods, and implicated in the control of the cardiovascular system and the muscles involved in feeding and gut motility. We aimed to determine the manifestation of this peptide family in the class Bivalvia. Acetone extracts of whole bivalves were fractionated by high pressure liquid chromatography (HPLC), and reactive peaks were identified by radioimmunoassay (RIA). After purification, sequencing, and analysis by mass spectroscopy, three peptides were identified in the clam *Mercenaria mercenaria:* IAMSFYFPRMamide, AMSFYFPRMamide, and YFAFPRQamide[4]. SCP-related peptides from two other species were also sequenced: APKYFYFPRMamide and SAFYFPRMamide from an oyster, *Crassostrea virginica;* and AMSFYFPRMamide (identical to one of the clam peptides) from a cockle, *Dinocardium robustum.* The tissue distribution and pharmacological actions of the clam SCPs were determined in *M. mercenaria,* as follows. The levels of peptide in extracts of 12 tissues were estimated by RIA. The largest concentrations of SCP occur in the palps and the visceral ganglia; the levels in the cerebral and pedal ganglia, the rectum, intestinal typhlosole, and gills were substantially lower; and the smallest amounts were found in the heart and the style sac typhlosoles. Immunohistochemistry revealed many cell bodies in the periphery of the ganglia and fibers in the neuropil. Immunoreactive, varicose fibers also occur in the typhlosoles of the intestine and style sac, and in the rectum, gill, and palps. The atrioventricular valves, but not the atria or ventricle proper, contain immunoreactive fibers. Synthetic clam SCPs were assayed on the rectum, the typhlosoles of the intestine and style sac, and the ventricle, all isolated in an organ bath. At low to moderate doses, the SCPs relaxed the muscles of the rectum; higher doses had biphasic actions. The muscles of the intestinal and style sac typhlosoles were relaxed, and spontaneous rhythmicity was slowed by the SCPs. Most ventricles were unresponsive. We conclude that the SCPs isolated in bivalves—though distinctive—are true homologs of those in gastropods. Moreover, the bivalve peptides also serve similar roles, controlling feeding and digestion, and perhaps even cardioactivity.

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[3] The logogram 'SCP,' its plural 'SCPs,' and the phrase 'SCP-related peptides' refer to a well-studied family of molluscan peptides. The initials were originally the abbreviation of the phrase 'small cardioactive peptides,' a designation for a peak of cardioexcitation in ganglion extracts of *Helix aspersa* that eluted relatively late from Sephadex G15 (Lloyd, 1978; discussed at length in Price *et al.,* 1990). Although the logogram SCP is widely used and recognized, the phrase 'small cardioactive peptides' no longer describes accurately the diverse functions of this peptide family or even the size of its members. To avoid confusion—since this paper is primarily *not* about cardioactivity—we avoid reference to the original abbreviation in the text or the title. Of course, the original meanings of names or symbols commonly lose their relevance and usage, in science (*e.g.,* substance P) as well as other endeavors (*e.g.,* ITT).

[4] The one-letter abbreviations of the amino acids are used to display all peptide sequences: A = Ala; D = Asp; E = Glu; F = Phe; G = Gly; I = Iso; K = Lys; L = Leu; M = Met; N = Asn; P = Pro; Q = Gln; R = Arg; S = Ser; W = Trp; Y = Tyr.

Introduction

Several years ago, two SCPs were isolated from *Aplysia californica* and *A. brasiliana,* respectively, and sequenced: SCP_A (ARPGYLAFPRMamide) (Lloyd *et al.,* 1987), and SCP_B (MNYLAFPRMamide) (Morris *et al.,* 1982). A genetic analysis in *A. californica* showed that both peptides

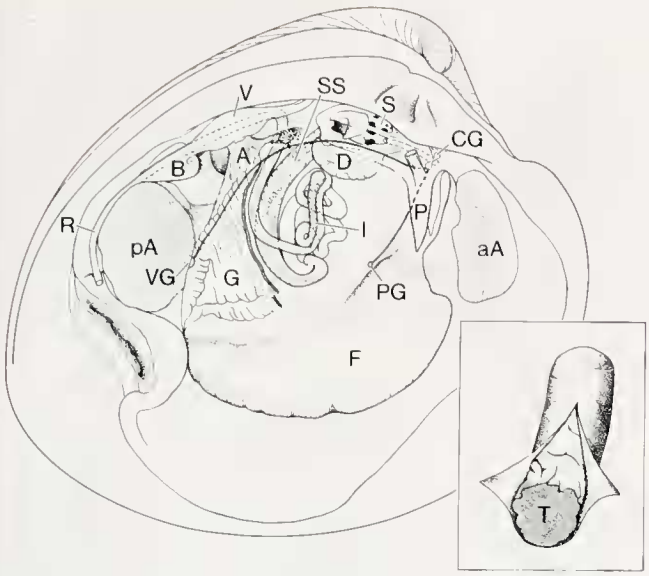

Figure 1. A diagram of *Mercenaria mercenaria* on the half-shell. The tissues, and particularly the parts of the mid-gut, utilized in these experiments are indicated. The segment of the intestine (I) that was used is stippled; the inset is an enlargement of that segment showing the typhlosole (T) within. The nervous system of this clam was thoroughly described by Loveland (1963). Abbreviations: A, atrium; aA, anterior adductor muscle; B, bulbus arteriosus; CG, cerebral ganglion; D, digestive gland; F, foot; G, gill (mostly cut away); I, intestine; P, palp; pA, posterior adductor muscle; PG, pedal ganglion; R, rectum; S, stomach; SS, style sac; T, typhlosole; V, ventricle; VG, visceral ganglion.

are processed from a common precursor (Mahon *et al.*, 1985). More recently, a pair of SCPs was also found in *Helix aspersa:* one of them is SCP_B, and the other is the

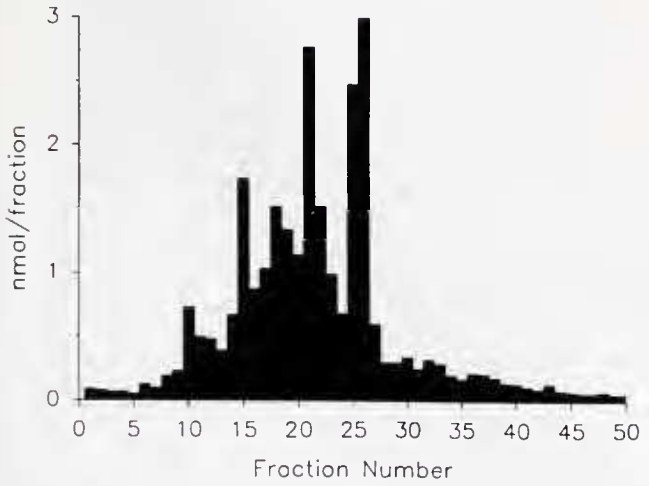

Figure 2. Immunoreactivity profile from the initial HPLC fractionation of a whole-clam extract. The extract was loaded onto a Prep-10 Octyl column (10 × 100 mm, 4 ml/min) and eluted with a gradient of acetonitrile (16–40% over 30 min) in water with 0.1% trifluoroacetic acid. Fractions were collected every half minute.

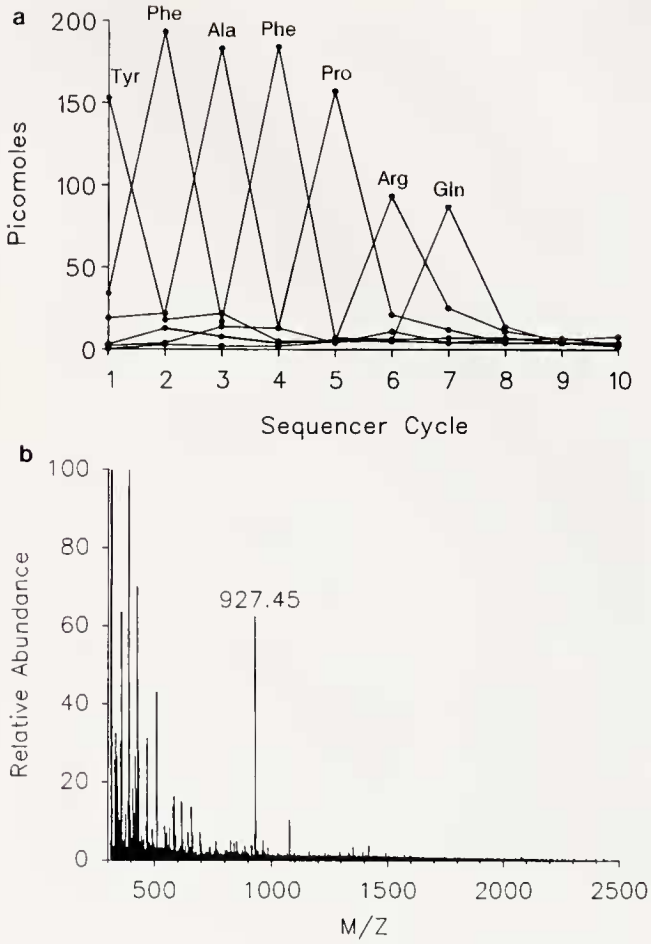

Figure 3. Identification of YFAFPRQamide from the earliest eluting SCP-like immunoreactive peak (fraction 10) in Figure 2. a. The yields of the pertinent amino acid derivatives at each cycle are plotted, and the most abundant amino acid is identified. b. FAB mass spectrum of this peak with the observed 927.45 molecular ion.

nonapeptide SGYLAFPRMamide (Price *et al.*, 1990). All of these peptides, as well as others from gastropods (Price *et al.*, 1989), have a common heptapeptide amide (−YLAFPRMamide) at the C-terminal.

The two SCPs from *Aplysia* have identical biological activities in that genus: they increase the amplitude and frequency of beat of the isolated heart (Lloyd *et al.*, 1985), regulate gut motility (Lloyd *et al.*, 1988), and enhance the amplitude of contraction of the accessory radula closer muscle, which is involved in biting (Lloyd *et al.*, 1984; Richmond *et al.*, 1986; reviewed by Weiss *et al.*, 1992).

The heart of the pulmonate snail *Helix aspersa* (Morris *et al.*, 1982; Price *et al.*, 1990) and that of the terrestrial slug *Limax maximus* (Prior and Welsford, 1989) are also potently stimulated by the SCPs. SCP_B also stimulates the isolated esophagus of the snail (Morris *et al.*, 1982) and, indeed, most studies of the SCPs in pulmonate snails and slugs (as in *Aplysia* and other opisthobranchs) have fo-

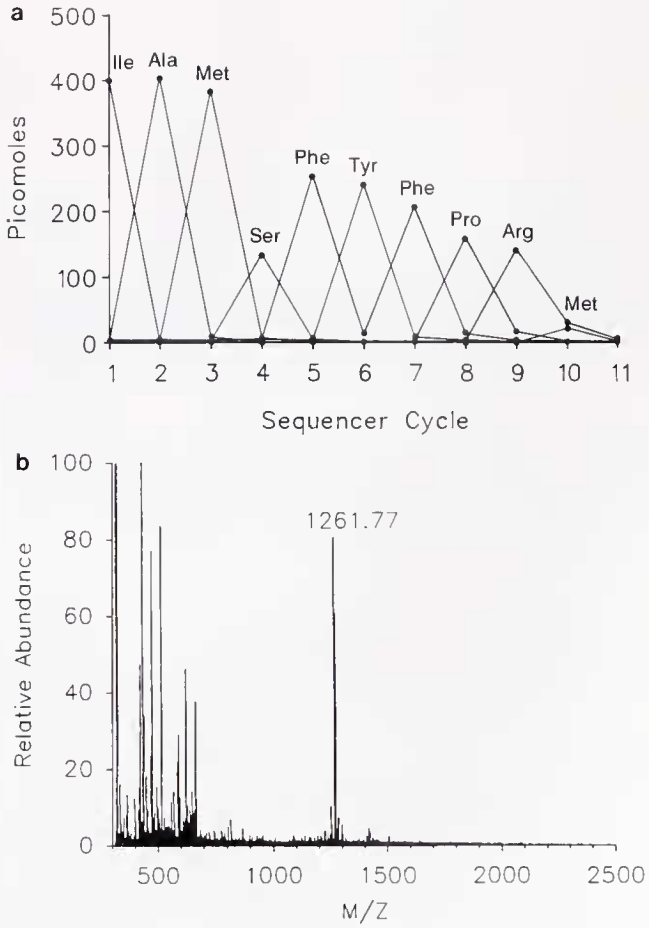

Figure 4. Identification of IAMSFYFPRMamide from the most retained SCP-like immunoreactive peak (fractions 25–27) in Figure 2. a. The levels of pertinent amino acid derivatives at each cycle are plotted, and the amino acid assigned to each position is shown. b. FAB mass spectrum of this peak with the prominent molecular ion 1261.77.

cused on the stimulation of feeding and gut motility (Murphy *et al.*, 1985; Lloyd and Willows, 1988; Willows *et al.*, 1988; Prior and Welsford, 1989; and Krajniak *et al.*, 1989).

We sought to learn whether, and to what extent, the structure and functions of the SCPs are retained in the Bivalvia, a class of mollusks that has been diverging from the Gastropoda since the early Cambrian (Pojeta *et al.*, 1973). We report here the isolation and sequencing of SCP analogs from the venerid clam, *Mercenaria mercenaria*, from another heterodont, the giant Atlantic cockle *Dinocardium robustrum*, as well as from a pteriomorph, the eastern oyster *Crassostrea virginica*. In *M. mercenaria*, we have also determined the tissue distributions of the clam peptides and have demonstrated their actions on the musculature of several isolated organs. Preliminary reports of these data have been published (Price *et al.*, 1989; Candelario *et al.*, 1990a; Candelario *et al.*, 1990b).

Materials and Methods

Animals

Quahogs (*Mercenaria mercenaria*) and eastern oysters (*Crassostrea virginica*) were obtained from the inland waters adjacent to Marineland, Florida. Giant cockles (*Dinocardium robustum*) were obtained from the beach in the same locality. The bivalves were maintained in flowing, natural seawater until they were needed, except that only freshly collected animals were used for immunohistochemistry.

Peptide characterization

Between 1 and 10 whole shucked animals, or batches of 10 or 20 pieces of particular tissues, were prepared for high pressure liquid chromatography (HPLC) as described previously (Price *et al.*, 1990). The only exception to the published procedure is that the acetone extraction was limited to overnight (about 16 h) to minimize oxidation;

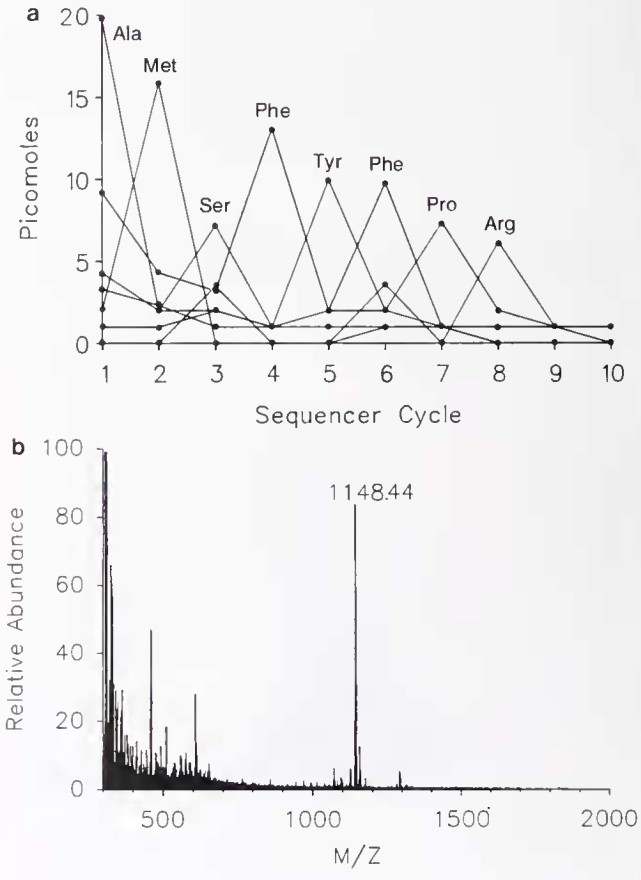

Figure 5. Identification of AMSFYFPRMamide from the fourth peak (fractions 21–22) in Figure 2. a. The levels of pertinent amino acid derivatives at each cycle are plotted, and the amino acid assigned to each position is shown. b. FAB mass spectrum of this peak with observed 1148.44 molecular ion.

Table I

Distribution of SCP-related immunoreactivity in tissues of the clam Mercenaria mercenaria

Tissue	p mole/g wet wt*
Palps (480, 450)	465
Visceral ganglia	426
Cerebral ganglia	250
Pedal ganglia	200
Gills (178, 170)	174
Rectum	170
Intestinal typhlosole	115†
Mantle (113, 96)	105
Foot (40, 30)	35
Adducter muscles (23, 22, 60)	35
Style sac typhlosole	9†
Heart	9

* Tissues from 10† or 20 animals were pooled, weighed, extracted in acetone, evaporated, and the aqueous portion fractionated by HPLC with analysis by RIA. The immunoreactivities of the peak fractions were added to produce the value indicated. Where more than one extract was tested, the means are reported in the right-hand column and the individual values are in parentheses.

i.e., the peptides were expected to be similar to gastropod SCPs and to contain multiple methionine residues.

After the acetone was removed and the extract filtered, the resulting clarified aqueous solution was loaded on a Prep10 Aquapore Octyl column (1.0 × 15 cm; Applied Biosystems) and eluted with an aqueous acetonitrile gradient containing trifluoroacetic acid (TFA; 0.1% throughout). The fractions were analyzed by radioimmunoassay (RIA; Price *et al.*, 1990). Each immunoreactive peak (consisting of one to three fractions) was further purified on a Spheri-5 RP18 (4.6 × 220 mm), or an Aquapore Octyl RP300 (2.1 × 220 mm) column. In addition to aqueous acetonitrile gradients, we also used aqueous isopropanol gradients, all containing 0.1% TFA.

In some cases, the fractions were treated with H_2O_2 (50 μl of 30%/ml) for 15 min to oxidize the methionyl residues of the peptides before re-chromatography (see Price *et al.*, 1990). In other cases, fractions were dried and incubated with dimethylsulfide (30 μl), TFA (50 μl), and trifluoromethanesulfonic acid (TMFSA; 10 μl) for 3 h (basically following the Applied Biosystems "low" conditions for TFMSA deprotection of synthetic peptides, but leaving out m-cresol) to reduce possible methionine sulfoxide residues. After the 3-h incubation, the fraction was diluted with deionized water and loaded onto the HPLC.

The purified peaks were analyzed by fast atom bombardment mass spectrometry (FABms; method in Bulloch *et al.*, 1988), by automated sequencing, and usually by both techniques. FABms and some sequencing was carried out at the Immunology Division of the Beckman Research Institute; most sequencing was done at the University of Florida Protein Core facility.

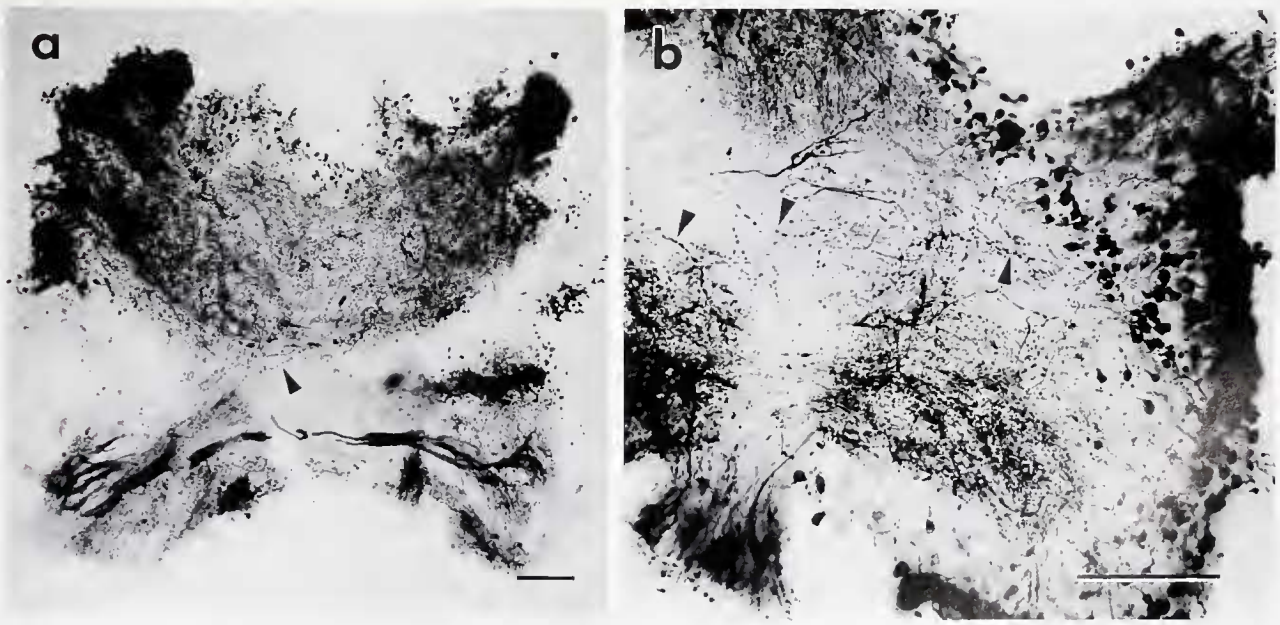

Figure 6. Frontal sections through the visceral (a) and cerebral (b) ganglia of *Mercenaria mercenaria* showing the distribution of neuronal cell bodies and fibers containing SCP-like immunoreactivity. The sections were stained by the PAP technique. Arrowheads indicate varicose fibers in the neuropil. Scale bars: 50 μm.

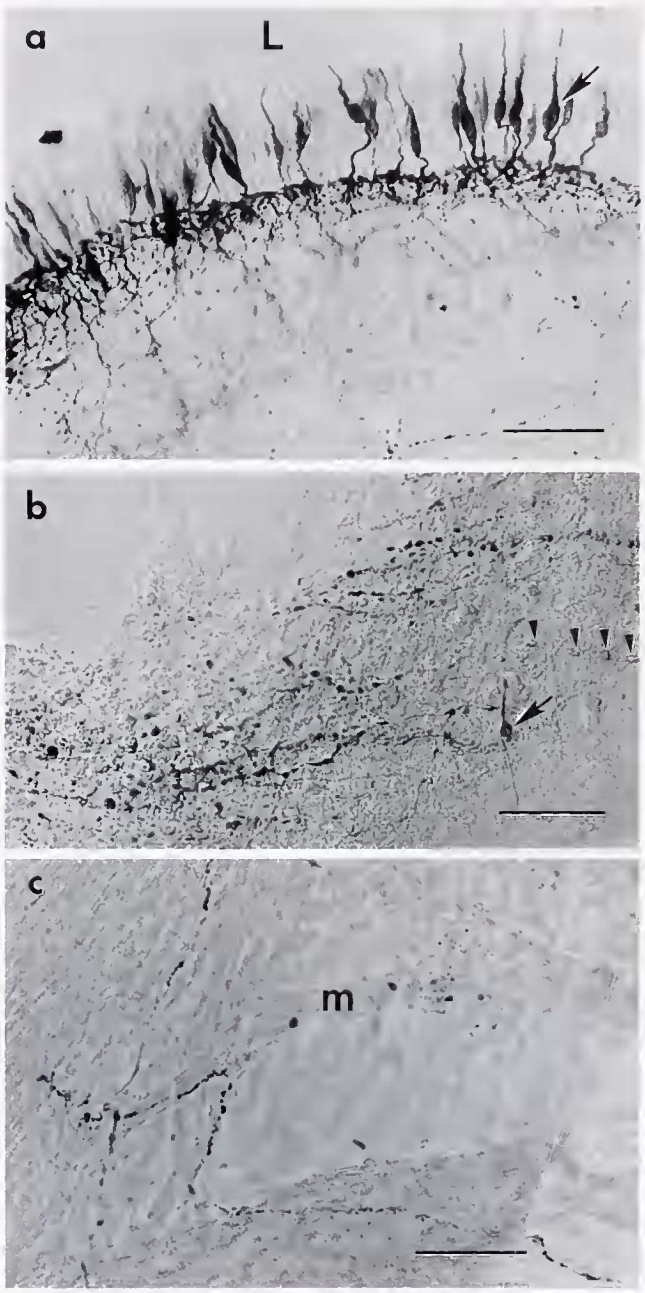

Figure 7. Differential interference contrast images of longitudinal sections through three regions of the digestive tract of *Mercenaria mercenaria*. The sections were stained by the PAP method. a. The surface of the style sac typhlosole shows SCP-like immunoreactivity within apparent sensory cells (arrow) that project toward the lumen (L). The bases of these cells join a network of varicose fibers, also stained. b. The wall of the intestine contains a network of varicose fibers and neuronal cell bodies (arrow) stained with SCP-like immunoreactivity. The network also includes cells that do not stain (arrowheads). c. Immunoreactive neural fibers in close association with muscle cells (m) in the wall of the rectum. Scale bars: 50 μm.

Immunohistochemistry

The three ganglia (visceral, pedal, and cerebral), as well as the rectum, the typhlosoles of the intestine and style sac, the gills, and the palps, were excised from *M. mercenaria*. The tissues were fixed and sectioned, as described below. They were then stained, either by indirect immunofluorescence or by the three-step peroxidase–anti-peroxidase (PAP) method (Beltz and Burd, 1989).

Immunofluorescence. The tissues were fixed in Bouin's solution, embedded in paraffin (Humason, 1967), and sectioned at 6–8 μm. The primary antibody, a monoclonal, was raised against SCP_B (Masinovsky *et al.*, 1988), and diluted 1:50 in a solution of 0.1 *M* phosphate buffered saline (PBS), 1% Triton X-100, and 3% normal goat antiserum at 4°C. The sections were incubated in this antibody solution for 12–18 h, rinsed with PBS for 30 min, and then stained with goat–anti-mouse IgG and IgM (both conjugated to fluorescein isothiocyanate) for 2 h at room temperature.

The PAP method. The protocol of Lesser and Greenberg (1993) was followed. The tissues were fixed overnight in 4% paraformaldehyde and then embedded in 12% gelatin dissolved in PBS. Sections (100 μm) were cut with a Vibratome (Series 1000; TPI, Inc.), and the gelatin was melted away. The sections were rinsed, and the nonspecific binding was blocked by treatment for 1 h with antibody diluent (3% Triton X-100, 5% normal goat serum, 3% bovine serum albumin in 0.1 *M* phosphate buffer). The sections were incubated overnight in primary antibody (1:50), rinsed, incubated overnight in goat–anti-mouse antiserum (1:100), rinsed again, incubated overnight in mouse-PAP (1:100), rinsed, incubated for 20 min in 0.5% DAB (3,3′-diaminobenzidine tetrahydrochloride; Sigma), and developed in H_2O_2. The reaction was quenched with phosphate buffer. The slides were dehydrated in a series of alcohols and xylene and mounted in Permount (Fisher) under coverslips.

Controls. Some sections were incubated with SCP_B antibody that had been preincubated with peptide for 24 h: *i.e.*, aliquots of antibody were individually preincubated with AMSFYFPRMamide (an endogenous clam peptide; see Results), with SCP_B, or with FMRFamide at 10^{-4} *M*. For some other sections, the SCP_B antibody was omitted from the primary incubation solution. The control sections were incubated with the secondary antiserum at the same time as the experimentals. Some sections were stained with a FMRFamide antiserum provided by Dr. E. Weber; this was a positive control.

Bioassays

Rectum. As described by Greenberg and Jegla (1963), the pericardial cavity was opened and the ventricular muscle teased away to expose the transcardiac segment

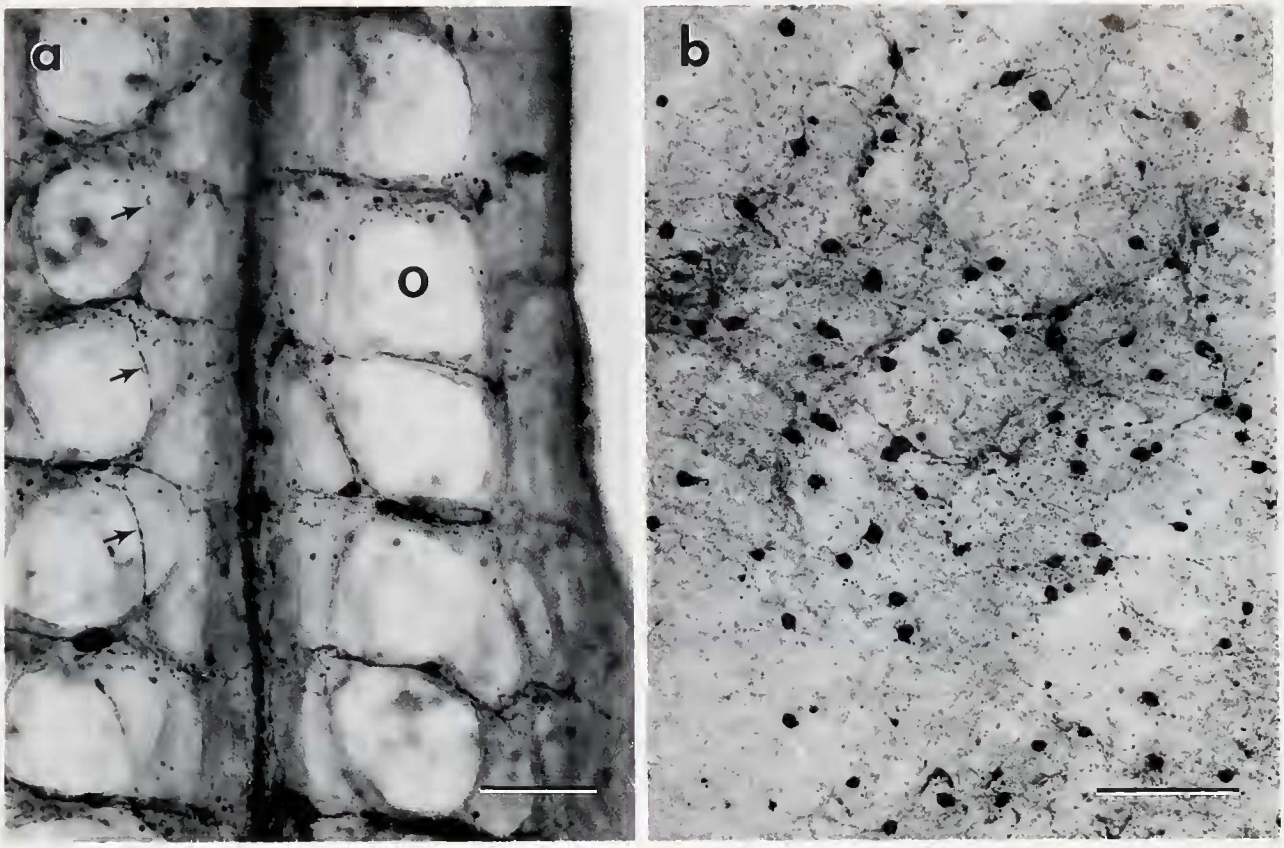

Figure 8. Frontal sections through the gill (a) and palp (b) of *Mercenaria mercenaria*. a. Gill: SCP-like immunoreactivity appears within neurons associated with the musculature of the ostia (O); note the iterated pattern of innervation (one example indicated by arrows). b. Palp: note numerous cell bodies and a dense network of varicose fibers containing SCP-like immunoreactivity. Scale bars: 50 μm.

of the rectum (Fig. 1). The muscular tube was ligated anterior to the bulbus arteriosus and posterior to the digestive gland, and was cut free distal to the ligations. The isolated rectum was suspended in a 5-ml aerated organ bath between a force transducer (Grass FT.03) and a stainless steel hook immersed in the bath. The tissue was superfused with natural seawater and aerated. Tension changes were recorded with an ink-writing oscillograph (Grass Model 7). Drugs were added directly to the bath; the doses are expressed as molar concentrations in the medium.

Typhlosoles of the intestine and style sac. These sections of the digestive tract both lie within the visceral mass of the clam (Fig. 1) and were prepared as follows. A shallow incision was made just through the epidermis on the right side of the visceral mass, from below the heart to the anterior adductor. Along this incision, the muscular body wall was peeled toward the foot, while being separated from the underlying adherent tissue. This procedure revealed two parallel segments of intestine covered with a thin layer of gonad, which was teased away. The wall of the intestine is extremely thin and delicate and ill-suited for bioassay. But when the intestinal wall was slit open, a large, firm typhlosole was revealed (Fig. 1 inset). A centimeter of this typhlosole was easily excised and the isolated tissue suspended in an organ bath, as described above for the rectum. We always used the typhlosole from the most posterior of the two parallel intestinal segments (Fig. 1).

The style sac contains two flaplike, longitudinal typhlosoles that separate it from the midgut. A segment of the sac close to the stomach was cut open, and the typhlosoles were removed, suspended in an organ bath, and used for bioassay.

Heart. The isolated heart of the clam was prepared according to the classical method of Welsh and Taub (1948).

Results

Peptide sequences

Mercenaria mercenaria. In the initial HPLC fractionation of clam extract, the immunoreactivity eluted between 5 and 15 min (fractions 10–30; Fig. 2); the active fractions were divided into 3 to 5 pools for further puri-

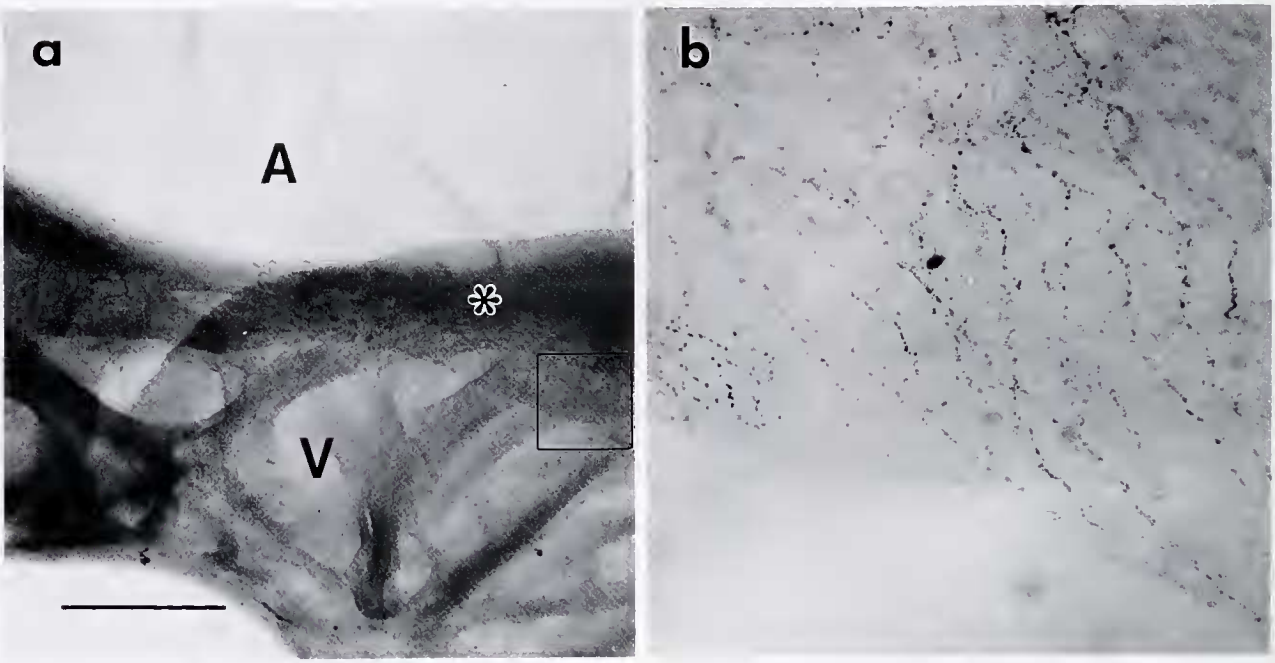

Figure 9. Wholemount of the atrioventricular region of the heart. (a) SCP-like immunoreactivity occurs only in varicose neuronal fibers in the valves (*); no immunoreactivity was seen in the atrial (A) or ventricular (V) musculature. (b) An enlargement of the boxed area in (a) showing varicose fibers in the valve. Scale bar: 500 μm.

fication. Most of these pools yielded more than one final pure peak of immunoreactivity. All of the final peaks were analyzed by FABms, and the most abundant peaks were sequenced as well. Two distinct sequences were found: YFAFPRQ and IAMSFYFPRM. The sequence YFAFPRQ (Fig. 3a) was obtained from the earliest eluting peak of immunoreactivity (fraction 10 in Fig. 2). It was associated with a 927.45 molecular ion (Fig. 3b), in good agreement with the value calculated for YFAFPRQamide (927.44); this indicated that the peptide is present as the amide and not the free acid.

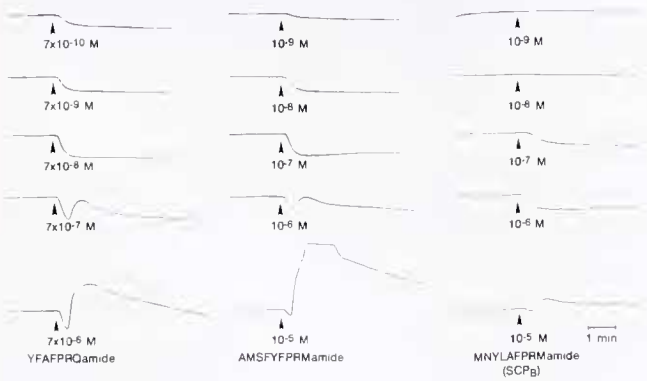

Figure 10. Dose-dependent responses of isolated clam rectums to two SCPs from the clam and one from gastropods (SCP_B). Doses were delivered at the arrowheads.

The sequence IAMSFYFPRM (Fig. 4a) is primarily found in the most retained peak (fractions 25–27 in Fig. 2), which contains the unoxidized form (molecular ion 1261.77; Fig. 4b). But oxidized forms (molecular ions of 1277.78 and 1293.75) can be isolated from the earlier peaks (especially fractions 18 and 19 in Fig. 2). AMSFYFPRM, a truncated version of IAMSFYFPRM, was sequenced (Fig. 5a) from the fourth peak (fractions 21 and 22 in Fig. 2; molecular ion, 1148.44, Fig. 5b); it also occurs in earlier peaks in oxidized forms (molecular ions, 1164.52 and 1180.44). Three ions corresponding to very low levels of even shorter forms were found in the two earliest peaks: 1077.56, MSFYFPRMamide; 946.47, SFYFPRMamide; and 859.51, FYFPRMamide. These three peptides are probably artifacts arising during purification. All of the ions associated with the series of peptides related to IAMSFYFPRMamide correspond to the amidated forms rather than the free acids.

Dinocardium robustum. Only one purified peak from this cockle was analyzed, and it yielded the sequence AMSFYFPRM. The associated molecular ion (1148.37) was in good agreement with that calculated for AMSFYFPRMamide (1148.54)—one of the peptides found in *M. mercenaria*. As in the clam, molecular ions for all fragments of this peptide, down to oxidized FYFPRMamide (875.49), were identified in *D. robustum*. Furthermore, a molecular ion of 927.46 was found, sug-

gesting that *Dinocardium*—like *Mercenaria*—contains YFAFPRQamide. The possibility that the longer *Mercenaria* peptide (IAMSFYFPRMamide) also occurs in *Dinocardium* has not been examined.

Crassostrea virginica. Two immunoreactive peaks were recovered from this oyster, and they contained material enough for both sequence and FABms analyses. Two distinct sequences were obtained, and their associated molecular ions indicated that the peptides are amidated: *i.e.*, APKYFYFPRMamide (1318.81) and SAFYFPRMamide (1017.58).

Distribution

Acetone extracts from the different tissues of the clam were fractionated by HPLC and analyzed for SCP-related peptides by RIA. The elution patterns of the immunoreactivity were essentially those seen in the whole animal, and the same peptides were present, as deduced by FABms. The amount of immunoreactivity varied from tissue to tissue, and the values for each could be sorted roughly into four classes (Table I). The visceral ganglia and palps contained the highest levels, and they were substantially higher than those of the cerebral and pedal ganglia, the rectum, and the gills. Some tissues had very low levels of SCP-like immunoreactivity, and the heart was among them.

Immunohistochemistry

Central ganglia. The cortex of the visceral, cerebral, and pedal ganglia, particularly around the bases of the connectives and nerves, contain many immunoreactive ovoid cell bodies (about 15×10 μm). In all three ganglia, a meshwork of stained fibers, some varicose, is present in the central neuropil; the density of these fibers is not uniform (Fig. 6).

Peripheral structures. Immunoreactive fibers, some of them varicose, are present in all of the tissues examined: the style sac typhlosole (Fig. 7a), intestinal typhlosole (Fig. 7b), rectum (Fig. 7c), gill (Fig. 8a), and palps (Fig. 8b). In the rectum, particularly, these fibers were very clearly associated with the musculature (Fig. 7c). A few small cell bodies (about 5 μm) were also observed in the periphery (*e.g.*, Figs. 7c, 8b). Immunoreactive fibers in the heart were restricted to the atrioventricular valves (Fig. 9a, b).

Controls. When the primary antibody was either not applied or was preincubated with AMSFYFPRMamide or SCP_B, staining was abolished. Staining persisted, however, when the primary antibody was preincubated with FMRFamide. The controls were the same for both staining methods.

Bioassay

The three novel peptides identified in *M. mercenaria* were synthesized and tested for their effects on isolated rectums and hearts of the clam. The effects of IAMSFYFPRMamide were indistinguishable from those of AMSFYFPRMamide, so only the latter peptide and YFAFPRQamide were tested on the typhlosoles of the intestine and style sac.

Rectum. Sixteen preparations were tested, most of them quiescent. The effect of low to moderate doses of the clam SCPs was a prompt relaxation of the rectum (Fig. 10); the threshold was 10^{-10}–10^{-9} *M*. When higher doses (10^{-6}–10^{-5} *M*) were applied, a biphasic response (*i.e.*, relaxation followed by contraction) was observed. Compared with the actions of AMSFYFPRMamide or its Ile^1 analog, YFAFPRQamide produced a stronger relaxation and a weaker contraction. SCP_B, the gastropod peptide, also decreased the tone of the rectum, but was much less potent than the endogenous bivalve peptides; threshold was about 10^{-8} *M* (Fig. 10).

Since acetylcholine (ACh) also relaxes the clam rectum with a threshold of 3×10^{-9}–3×10^{-8} *M* (Greenberg and Jegla, 1963), the SCPs might be acting presynaptically to release ACh. But the addition of 10^{-5} *M* benzoquinonium chloride (an ACh antagonist) to the bath did not alter the response of the rectum to the SCPs.

The effect of 5-HT (10^{-6} *M*) on the clam rectum is a large contracture, sometimes accompanied by rhythmic "beating" (Greenberg and Jegla, 1963; Doble and Greenberg, 1982). In five experiments, the SCP-like peptide YFAFPRQamide was applied after the effect of 5-HT had developed (Fig. 11). Under these conditions, YFAFPRQamide usually relaxed the 5-HT contracture, and then either augmented the rhythmical contractions or reduced them. These combined actions were extraordinarily variable.

Intestinal typhlosole. We tested 18 strips of intestinal typhlosole, and unlike the rectum, all displayed slow, regular, rhythmical activity with a frequency of 2–7 contractions per minute. The effects of the two SCPs tested (AMSFYFPRMamide and YFAFPRQamide) were similar: both reduced the frequency and force of contraction, as well as the tone of the preparation (Fig. 12). YFAFPRQamide seems to be more potent; its threshold is 10^{-8} *M* compared with 3×10^{-7} *M* for AMSFYFPRMamide.

In preliminary experiments, 5-HT increased both the basal tone of the preparation and the frequency of contractions (threshold, about 3×10^{-7} *M*); FMRFamide had a similar effect, but at a slightly higher threshold (10^{-6} *M*); and ACh seemed to have a biphasic effect, as it does on the rectum, but never at a dose below 3×10^{-6} *M*. These effects were modest, except at very high concentrations.

Style sac typhlosoles. Of the eight preparations tested, six exhibited rhythmic activity, particularly after the application of peptides. Of the two SCPs tested,

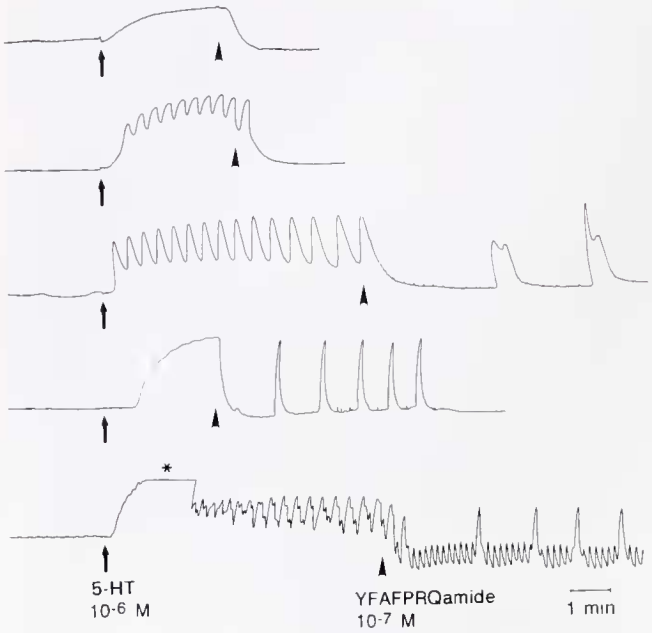

Figure 11. Responses of isolated clam rectums to YFAFPRQamide in the presence of 5-hydroxytryptamine (5-HT). The preparations were first stimulated with 5-HT (arrow). When the effect was established (contracture with or without rhythmical activity), the peptide was added (arrowhead). *Recording off-scale; baseline adjusted.

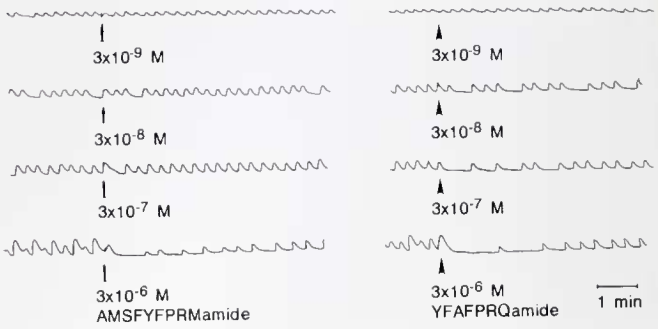

Figure 12. Dose-dependent effects of two clam SCPs on the isolated typhlosole of the clam intestine. Doses were delivered at the arrows or arrowheads.

YFAFPRQamide was again more potent: it inhibited rhythmicity transiently at 10^{-9} *M*, and relaxed the tissue at 10^{-8} *M*. These effects were also produced by AMSFYFPRMamide, but at doses about 3- to 10-fold higher (Fig. 13).

Heart. The effects of the SCP-related peptides were tested on 42 isolated hearts. More than half of the preparations were either not affected (10^{-7}–10^{-4} *M*), or an effect appeared that could not be repeated. The rest of the hearts responded reliably to the SCPs. The threshold action was an inhibition of beat amplitude (threshold, 10^{-7}–10^{-6} *M*). At higher doses, beat and tone increased transiently, and the heart was finally arrested, usually in diastole. Acceptable dose-response relationships were seen in only five or six experiments.

Discussion

We have isolated and sequenced three SCP-related peptides in the venerid clam *Mercenaria mercenaria:* IAMSFYFPRMamide, AMSFYFPRMamide, and YFAFPRQamide. The latter two peptides were also identified in the cockle *Dinocardium robustum.* But two quite different SCP-like sequences were found in the eastern oyster *Crassostrea virginica* (Table II). The features of these bivalve peptides are best evaluated with reference to an SCP-like sequence found in a mussel, *Mytilus edulis* (Fujisawa *et al.*, 1993), as well as six sequences identified in various gastropods (Lloyd *et al.*, 1987; Price *et al.*, 1989; Price *et al.*, 1990). The total set includes 12 peptides from 13 species (Table II).

The bivalve SCPs as a group, including peptides from both pteriomorphs and heterodonts, are clearly distinct from the gastropod peptides. All but one of the gastropod SCPs—from a selection of species representing all three subclasses—contain the C-terminal heptapeptide -YLAFPRMamide, and the substitution of an isoleucyl residue for a leucyl in *Littorina irrorata* is a conservative one. In contrast, the bivalve peptides are much more diverse, and none of them has fewer than two substitutions with respect to the gastropod C-terminal heptapeptide. Indeed, in two of these peptides—YFAFPRQamide (in *M. mercenaria*) and APNFLAYPRLamide (in *M. edulis*)—the terminal methionine is replaced.

The high pharmacological potency of YFAFPRQamide, and the similarity of its effect on the clam gut to that of AMSFYFPRMamide and the gastropod peptide

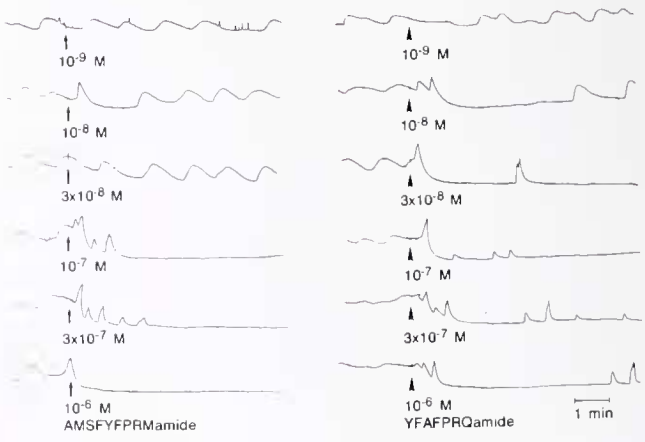

Figure 13. Dose-dependent responses of isolated segments of the style sac typhlosole to two clam SCPs. Doses were delivered at the arrows or arrowheads.

Table II

Sequences and species distribution of known molluscan SCPs

CLASS SEQUENCES[a]	Subclass *Species*[b,c]		
BIVALVIA	Pteriomorphia	Heterodonta	
A P N **F L A Y P R L** a	*Med*[1]		
A P K **Y F Y F P R M** a	*Cvi*		
S **A F Y F P R M** a	*Cvi*		
I A M **S F Y F P R M** a		*Mme*	
A M **S F Y F P R M** a		*Mme, Dro*	
Y F A F P R Q a		*Mme, Dro*	
GASTROPODA	Prosobranchia	Opisthobranchia	Pulmonata
S G **Y L A F P R M** a			*Has.*[2] *Lst.*[3] *Spe.*[3] *Htr.*[3] *Bgl.*[3]
M N **Y L A F P R M** a		*Abr.*[4] *Aca*[5]	*Has.*[2] *Spe*[3]
S Q G **Y L A F P R M** a			*Htr.*[3] *Bgl*[3]
A R P G **Y L A F P R M** a		*Aca*[6]	
S Q P **Y I A F P R M** a	*Lit*[3]		
N **Y L A F P R M** a	*Tha*[3]		

[a] The residues in the standard gastropod C-terminal heptapeptide (-YLAFPRMa) are in bold italics; residues that are different from the standard are underlined. a = amide.

[b] Species abbreviations: first letter of genus plus first two letters of species:

Abr, Aplysia brasiliana
Aca, Aplysia californica
Bgl, Biomphalaria glabrata
Cvi, Crassostrea virginica
Dro, Dinocardium robustum
Has, Helix aspersa
Htr, Helisoma trivolvis
Lit, Littorina irrorata
Lst, Lymnaea stagnalis
Med, Mytilus edulis
Mme, Mercenaria mercenaria
Spe, Siphonaria pectinata
Tha, Thais haemastoma

[c] References:
[1] Fujisawa *et al.*, 1993
[2] Price *et al.*, 1990
[3] Price *et al.*, 1989
[4] Morris *et al.*, 1982
[5] Mahon *et al.*, 1985
[6] Lloyd *et. al.*, 1987

SCP_B (MNYLAFPRMamide; Table II), suggest that the substitution of a Gln for a Met at the C-terminal carries little apparent functional penalty. Further, the structural features critical for the function of the clam SCPs seem not to be confined to the C-terminal. Notwithstanding the high potency of YFAFPRQamide on clam gut, Gln may not be an effective replacement for Met in general. Indeed, very high doses of YFAFPRQamide are required for activity on the heart of *Helix aspersa*, (W. Lesser, personal communication), whereas SCP_B is a highly potent excitor of this preparation (Price *et al.*, 1990). So the structure-activity relations of the gastropod heart may be very different from those of the bivalve gut.

In *M. mercenaria*, the number of SCP-like peptides that are primary products of precursor processing remains uncertain. We have isolated and sequenced three peptides; but no more than two SCPs have been found in any other species (Table II), and the preprohormone in *Aplysia californica* includes only one copy each of SCP_B and SCP_A (Mahon *et al.*, 1985). Furthermore, two of the peptides we have identified (IAMSFYFPRMamide and AMSFYFPRMamide) differ only by the occurrence or lack of a single isoleucine residue at the N-terminal. Thus, the shorter peptide, AMSFYFPRMamide, might well be a degradation product. Indeed, we have identified, by their molecular ions, a series of peptides representing successive deletions at the N-terminal down to FYFPRMamide. Still, the SCP precursor of *Aplysia* is the only one known, so we cannot rule out the possibility that both IAMSFYFPRMamide and AMSFYFPRMamide are, with YFAFPRQamide, primary processing products of one or more preprohormones. This issue will only be resolved when the mRNAs encoding the clam or cockle precursors have been identified and sequenced.

The distribution and pharmacological actions of a peptide are often indicative of its role in the animal. For example, the visceral ganglia, containing roughly twice as much SCP as either the cerebral or pedal ganglia, innervate the posterior sections of the mantle, the somatic musculature, and the gut, as well as the gills and the renopericardial organs (Loveland, 1963). Of these potential targets, the gills, intestine, and rectum contain about a quarter of the amount of SCP in the visercal ganglia. Moreover, immunoreactive fibers are present in the rectal musculature, the intestinal typhlosole, and the gills. Finally, the clam SCPs are also biologically active, not only on the gut musculature, as reported here, but on the cilia of isolated gill demibranchs (L. F. Gainey, Jr., personal communication). These findings suggest that one role of the SCPs in *M. mercenaria* is to regulate feeding and gut motility, as in gastropods (see Prior and Welsford, 1989; Lloyd, 1989; and Weiss *et al.*, 1992).

Other data are less supportive of this notion, or are incomplete. The style sac typhlosoles contain very low levels of peptide, yet immunoreactive neural fibers are clearly evident, and the SCPs reduce rhythmic activity and relax the tissue at low doses. In contrast, the palps, which are innervated from the cerebral ganglia, contain very high levels of SCPs and, again, the peptide has been localized to a network of neurons. But the palps are complex organs, with both sensory and motor (muscular, ciliary, and secretory) functions. In the absence of an appropriate bioassay, the roles of the SCPs in this tissue remain enigmatic.

The pharmacology of the bivalve midgut has never before been examined, and rhythmical muscular activity of the typhlosole has not previously been reported. Indeed, the notion that digested food and feces are moved through the midgut by cilia is widely accepted (*e.g.*, Morton, 1983). Our results suggest that the typhlosole plays a role in moving material through the gut. The relative ease with which this tissue is prepared and the regularity of its contractions suggest that further studies of its regulation will be possible.

Belying the original meaning of 'SCP' (see footnote 3), the clam heart contains very low levels of SCP-related immunoreactivity and is poorly responsive to the synthetic peptides, with a threshold at least 100 times higher than those of gastropod hearts. But nerve fibers containing SCP-related immunoreactivity do occur in the atrioventricular valves, and isolated valves treated with YFAFPRQamide contract and begin to beat rhythmically (N. A. Pennell and W. Lesser, personal communication). In conclusion, the SCPs may play a cardioregulatory role in the clam, perhaps limited to the valves, but certainly unlike that in *Helix aspersa* (Price *et al.*, 1990; Lesser and Greenberg, 1993) or *Aplysia californica* (Lloyd *et al.*, 1985; Skelton *et al.*, 1992).

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